

KARYOTYPE ANALYSIS IN SEVERAL PELAGIC GASTROPODS

CATHERINE THIRIOT-QUIÉVREUX
UNIVERSITÉ P. ET M. CURIE
STATION ZOOLOGIQUE
06230 VILLEFRANCHE-SUR-MER, FRANCE

ABSTRACT

Chromosome number and morphology were determined for six species of pelagic gastropods: the Thecosomata *Hyalocylis striata* Rang (2n=28); the Gymnosomata *Pneumodermopsis paucidens* Boas (2n=32) and *Paraclione longicaudata* Souleyet (2n=32); the Heteropoda *Pterotrachea coronata* Forskal (female 2n=32, male 2n=31), *Pterotrachea hippocampus* Philippi (male 2n=31) and *Firoloida desmaresti* Lesueur (female 2n=32, male 2n=31). The three latter species displayed male karyotypes with heteromorphic chromosomes which suggest a XY₁Y₂ sex mechanism. Comparison of known karyological data among the Thecosomata shows an evolutionary trend from low diploid complement with metacentric chromosomes [as in *Limacina inflata* (Orbigny)] toward higher diploid complement and an increase in the proportion of subtelocentric-telocentric chromosomes (as in *Cymbulia peroni* De Blainville). Within the Gymnosomata, the known four species reveal a chromosomal stability in the chromosome number and a variable proportion of metacentric-submetacentric and subtelocentric-telocentric chromosomes. The chromosome diploid number in Heteropoda ranges close to the diploid complement found in Hydrobiidae, Littorinidae and Naticidae. The unusual sex-determining mechanism XY₁Y₂ is discussed among the Mesogastropoda.

Chromosomal data on pelagic gastropods have been limited to the knowledge of the chromosome number for five species of pteropods (Nakrassov, 1904; Zarnick, 1911) and two species of heteropods (Boveri, 1890). The development of cytogenetics has yielded karyological investigations in many molluscan species (e.g. see reviews by Nakamura, 1985, 1986), but none on pelagic gastropods. Yet, the holoplanktonic animals are of particular interest among gastropods in illustrating the evolution of unusual adaptation to the pelagic environment. Furthermore, three taxonomical groups of pelagic gastropods, i.e. two opisthobranch orders, Thecosomata and Gymnosomata, and one prosobranch group, Heteropoda, show important anatomical variations within their groups. The phylogenetic origin of these taxons remains obscure and have led to considerable speculation (Lalli and Gilmer, 1989). Cytogenetic parameters such as chromosome number and morphology can also be used as an investigatory tool to trace phylogenetic patterns and evolutionary relationships. With this aim in mind, chromosome studies on seven species of Thecosomata and two species of Gymnosomata were first carried out (Thiriot-Quévieux, 1988). In the present paper, chromosome investigations are added for one species of Thecosomata, two species of Gymnosomata, and three species of Heteropoda. Evolutionary cytotaxonomy and karyological tendencies of these taxons are discussed.

MATERIALS AND METHODS

Pelagic gastropods examined in this paper were collected from plankton tows taken at 20 m in the bay of Villefranche-sur-Mer (Mediterranean coast of France). The following species were investigated: Thecosomata Euthecosomata Cavoliniidae: *Hyalocylis striata* Rang; Gymnosomata Pneumodermatiidae: *Pneumodermopsis paucidens* Boas; Gymnosomata Clionidae: *Paraclione longicaudata* Souleyet; Heteropoda Pterotracheidae: *Pterotrachea coronata* Forskal, *Pterotrachea hippocampus* Philippi and *Firoloida desmaresti* Lesueur.

Chromosome preparations were from gonads during gametogenesis according to techniques described by Thiriot-Quévieux (1988). Staining was effected with Giemsa 4%, pH 6.8 and according to Howell and Black (1980) for NORs (nucleolar organizer region). Chromosome measurements and statistical interpretations were made with a CHROMOS program (Thiriot-Quévieux, 1984; Thiriot-Quévieux *et al.*, 1988).

Relative length (= percent total complement length) was expressed as the ratio of absolute chromosome pair length over total length of haploid complement x 100. Centromeric index was calculated as the ratio of length of short arm over total length of chromosomes x 100. Arm ratio was calculated as length of short arm/length of long arm.

Terminology relating to centromere position follows that of Levan *et al.* (1964).

RESULTS

THECOSOMATA: *HYALOCYLIS STRIATA*

At mitotic metaphases, the diploid number of chromosomes is $2n=28$. Figure 1 shows the karyotype arranged by decreasing chromosome size. Table 1 gives the measurements and classification of four selected metaphase plates. Pairs 1, 2, 3, 4, 9, 11 and 13 are metacentric, pairs 12 and 14 are submetacentric, pair 10 is subtelocentric-submetacentric (the centromere position being on the borderline between two different categories with 95% confidence limits), pairs 5, 6, 7 and 8 are subtelocentric. The ideogram (Fig. 2) constructed from relative length and centromeric index values visualizes the karyotype with the different morphological types of chromosomes.

GYMNOSOMATA: *PNEUMODERMOPSIS PAUCIDENS*

Mitotic metaphases were observed with a diploid set of $2n=32$. Eight selected spreads from different animals were karyotyped and measured (Table 2). In the karyotype (Fig. 3A),

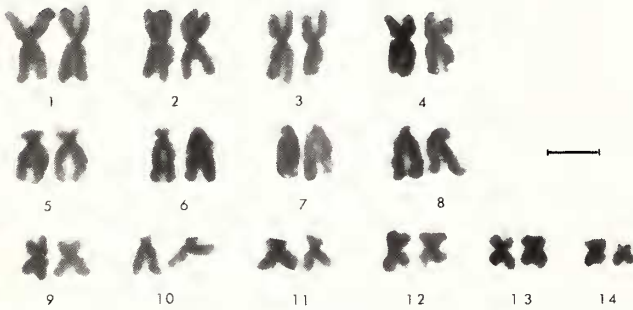


Fig. 1. Karyotype of *Hyalocylis striata* (scale bar = 5 μ m).

the first metacentric pair is about five times longer than the last pair and conspicuously larger than the remaining pairs whose size decrease progressively. Pairs 1, 2, 3, 4, 6, 12 and 14 are metacentric, pairs 8, 9, 10, 13, 15 and 16 are submetacentric, pair 11 is subtelocentric-submetacentric, and pair 7 telocentric.

Comparison of the same metaphase stained with standard Giemsa (Fig. 4C) then silver-stained (Fig. 4D) showed that the NORs are located on the metacentric pair 2. Silver-stained metaphases in *Pneumodermopsis canephora* Pruvot-Fol (Fig. 4A) and *Pneumoderma atlanticum* Oken (Fig. 4B) showed NORs on the short arm of the subtelocentric pair 2.

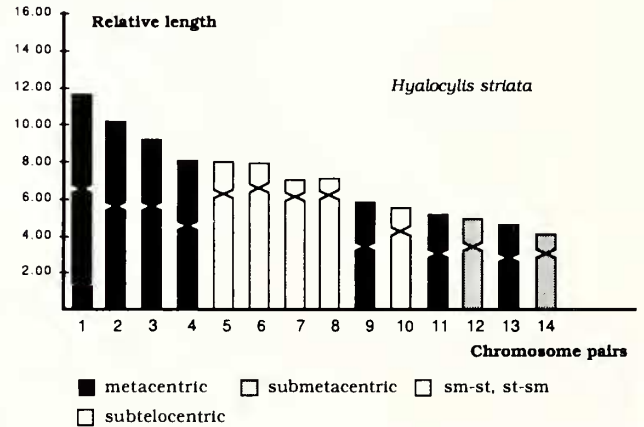


Fig. 2. Ideogram of *Hyalocylis striata* constructed from relative length and centromeric index values. Chromosome morphology is indicated: dark stippled for metacentric, medium stippled for submetacentric, light stippled for subtelocentric-submetacentric or subtelocentric-submetacentric (i.e. $23.06 < Ci < 26.74$), open for subtelocentric.

Table 1. Chromosome measurements and classification in four metaphasic cells of *Hyalocylis striata*.

Chromosome pair no.	Relative length		Arm ratio		Centromeric index		Classification*
	Mean	SD	Mean	SD	Mean	SD	
1	11.61	0.813	0.796	0.037	44.15	1.232	m
2	10.30	0.339	0.848	0.151	44.84	3.840	m
3	9.29	0.366	0.675	0.087	39.51	3.175	m
4	8.02	0.455	0.834	0.146	44.82	4.707	m
5	8.07	0.434	0.297	0.090	22.40	5.616	st
6	7.92	0.437	0.213	0.085	17.03	5.487	st
7	7.12	0.442	0.149	0.020	12.87	1.556	st-t
8	7.01	0.367	0.150	0.055	12.78	4.132	st-t
9	5.97	0.165	0.724	0.131	41.23	4.452	m
10	5.49	0.149	0.311	0.089	23.30	5.114	st-sm
11	5.37	0.214	0.727	0.269	40.04	7.063	m
12	4.97	0.162	0.491	0.372	29.14	17.240	sm
13	4.70	0.425	0.785	0.222	41.77	4.586	m
14	4.11	0.364	0.381	0.052	27.15	2.712	sm

*m = metacentric; st = subtelocentric; st-t = subtelocentric-telocentric; st-sm = subtelocentric-submetacentric; sm = submetacentric.

Table 2. Chromosome measurements and classification in eight metaphasic cells of *Pneumoderopsis paucidens*.

Chromosome pair no.	Relative length		Arm ratio		Centromeric index		Classification*
	Mean	SD	Mean	SD	Mean	SD	
1	13.48	0.628	0.880	0.056	46.60	1.580	m
2	8.83	0.561	0.701	0.599	40.63	2.595	m
3	8.15	0.328	0.809	0.109	44.08	3.320	m
4	7.96	0.371	0.698	0.162	40.18	5.822	m
5	7.22	0.501	0.319	0.095	23.42	5.173	st
6	7.11	0.244	0.716	0.167	40.58	5.290	m
7	6.22	0.667	0.132	0.044	11.39	3.277	t
8	6.07	0.464	0.403	0.126	27.82	6.678	sm
9	6.06	0.479	0.633	0.264	36.50	8.493	sm-m
10	5.46	0.368	0.545	0.144	34.47	6.854	sm
11	5.37	0.510	0.322	0.204	22.62	10.820	st-sm
12	4.55	0.238	0.700	0.161	39.95	5.713	m
13	4.22	0.271	0.441	0.148	29.34	5.995	sm
14	3.90	0.239	0.692	0.129	39.93	3.940	m
15	2.90	0.252	0.420	0.079	28.84	4.012	sm
16	2.41	0.341	0.543	0.173	33.93	7.036	sm

*m = metacentric; st = subtelocentric; t = telocentric; sm = submetacentric; sm-m = submetacentric-metacentric; st-sm = subtelocentric-submetacentric.

GYMNOSOMATA: *PARACLIONE LONGICAUDATA*

Thirty-two chromosomes were counted in mitotic metaphases. Table 3 gives chromosome measurements on

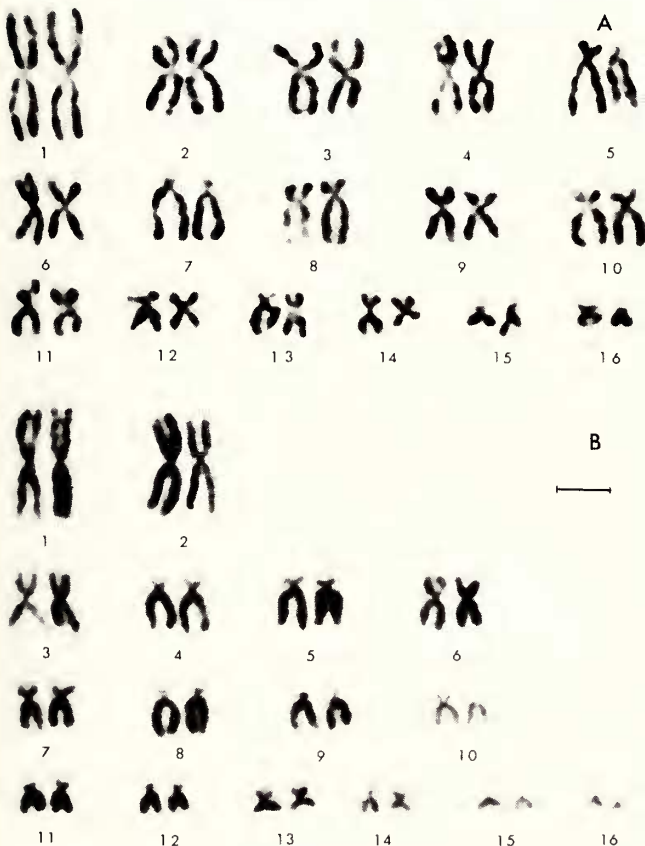


Fig. 3. Gymnosomata karyotypes (scale bar = 5 μ m). (A) *Pneumoderopsis paucidens*, (B) *Paraclione longicaudata*.

six selected spreads. The overall appearance of the karyotype (Fig. 3B), better visualized in the ideogram (Fig. 5), is very different from the previous species. Pair 1 represents the highest relative length of Gymnosomata species up to now investigated and is about nine times longer than the last pair. Pair 2 is also conspicuously larger than the remaining pairs whose size decrease progressively from pair to 14. Pairs 15 and 16 are very small (relative unit length 2.55 and 1.61, respectively). Morphology of the pairs is as follows: metacentric for pairs 1, 2, 3 and 6, submetacentric for pairs 7 and 13, subtelocentric-submetacentric for pair 14, subtelocentric for pairs 4, 5, 8, 9, 10, 11, 12, and telocentric for pairs 15 and 16. Metaphases were not successfully silver-stained in this species.

HETEROPODA

Due to the small number of individuals investigated in the following species, chromosome measurements were made only to determine chromosome morphology. Therefore, neither tables nor ideograms are given.

PTEROTRACHEA CORONATA

In this species, two females and one male were investigated. The female complement consists of $2n=32$. In nine cells studied, the female karyotype (Fig. 6A) shows two large metacentric, then five metacentric of decreasing size, then five submetacentric, one submetacentric-subtelocentric and one subtelocentric pairs of about the same size. For the male, the complement set consists of $2n=31$. In eight cells studied, the first large metacentric pair of the karyotype (Fig. 6B) is similar to that of the female. Next, heteromorphic chromosomes are present: a metacentric, a submetacentric-subtelocentric and a small telocentric suggesting the presence of sex chromosomes XY_1Y_2 . Thus, by comparison



Fig. 4. Comparison of nucleolar organizer regions (NORs) in three Gymnosomata species (scale bar = 5 μ m). (A) silver-stained mitotic metaphase of *Pneumodermopsis canephora* (arrows point to NORs), (B) silver-stained mitotic metaphase of *Pneumoderma atlanticum* (arrows point to NORs), (C) Giemsa-stained mitotic metaphase of *Pneumodermopsis paucidens* (arrows on pair 2), (D) same metaphase, silver-stained (arrows on NORs).

the pair 2 of the female could be called XX. The remaining pairs in the male karyotype are similar to the female except the absence of the smallest autosome pair.

PTEROTRACHEA HIPPOCAMPUS

One male could only be investigated in this species. Sixteen well-spread metaphases were analysed with a complement set of $2n=31$. The karyotype (Fig. 7) shows heteromorphic chromosomes, comprising a large metacentric X, a telocentric Y_1 and a minute chromosome Y_2 , and 14 autosome pairs with two metacentric, two submetacentric, one subtelo-centric-submetacentric, six subtelo-centric and three telocentric.

FIROLOIDA DESMARESTI

In this species, several individuals of females and males were studied. But only two metaphasic cells in females and four in males could be karyotyped because of overlapping chromosomes. The overall large absolute chromosome size (Fig. 8A, B) of this species is probably the reason for this overlapping.

The female diploid set consists of $2n=32$ while the male shows $2n=31$. In both sexes, the first pair is a large metacentric. Next, the second metacentric pair in the female corresponds to XX chromosomes while in the male sex heteromorphic chromosomes are observed comprising a large metacentric X, a large submetacentric-subtelocentric Y_1 and a microchromosome Y_2 . The remaining autosome pairs in the

Table 3. Chromosome measurements and classification in six metaphasic cells of *Paraclione longicaudata*.

Chromosome pair no.	Relative length		Arm ratio		Centromeric index		Classification*
	Mean	SD	Mean	SD	Mean	SD	
1	15.15	0.725	0.877	0.055	46.65	1.554	m
2	11.78	0.864	0.751	0.119	42.07	3.642	m
3	8.17	0.497	0.733	0.121	41.47	3.315	m
4	7.99	0.346	0.227	0.025	18.44	1.645	st
5	7.45	0.352	0.265	0.112	20.32	6.303	st
6	7.04	0.593	0.768	0.115	42.26	3.444	m
7	6.59	0.439	0.366	0.070	26.45	3.667	sm
8	5.82	0.512	0.165	0.081	13.79	5.809	st
9	5.11	0.272	0.266	0.113	20.03	6.663	st
10	4.67	0.285	0.233	0.102	18.30	6.660	st
11	4.36	0.396	0.270	0.092	20.78	5.006	st
12	4.10	0.092	0.195	0.094	16.07	6.287	st
13	3.91	0.466	0.510	0.097	32.73	3.035	sm
14	3.85	0.542	0.363	0.202	24.84	10.174	st-sm
15	2.55	0.233	0.088	0.021	8.06	1.809	t
16	1.61	0.425	0.141	0.721	11.65	5.230	t

*m = metacentric; st = subtelocentric; sm = submetacentric; st-sm = subtelocentric-submetacentric; t = telocentric.

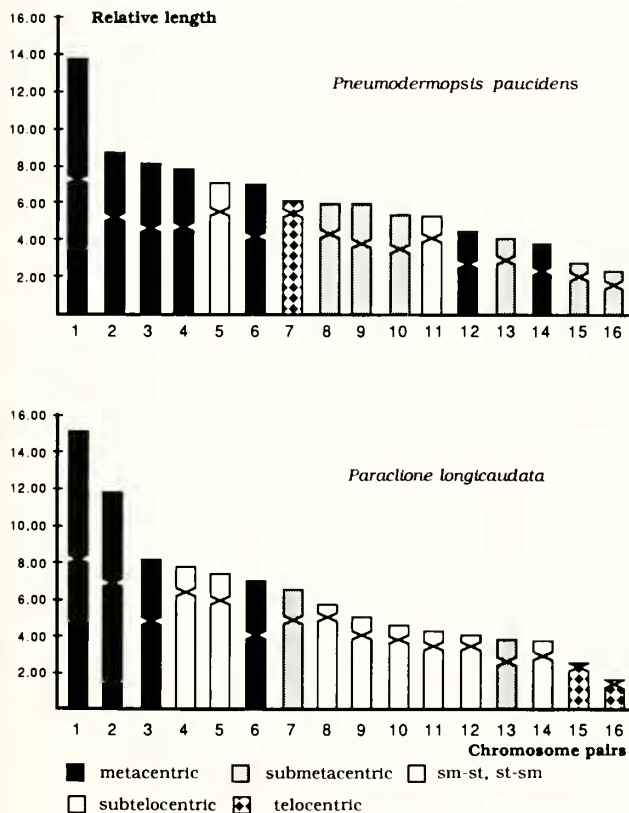


Fig. 5. Ideogram of *Pneumodermopsis paucidens* and *Paraclione longicaudata*, constructed from relative length and centromeric index values. Chromosome morphology is indicated: dark stippled for metacentric, medium stippled for submetacentric, light stippled for submetacentric-subtelocentric or subtelocentric-submetacentric, open for subtelocentric, lozenge stippled for telocentric.

female are four metacentric, four submetacentric, five subtelocentric and two telocentric. In the male, the smallest autosome pair is lacking.

DISCUSSION

THECOSOMATA

The diploid number of $2n=28$ observed in *Hyalocylis striata* is different from the number found by Zarnick (1911). This difference in counts for the same species may result from the difficulty in evaluating chromosome number using older cytological techniques. Comparing data obtained with the same techniques, however, points out that *H. striata* shows the highest diploid number among Euthecosomata.

Karyological data for all species studied in a previous paper (Thiriote-Quévieux, 1988) and in the present work are recapitulated in table 4. The chromosome morphology of *Limacina inflata* (Orbigny), *Peraclis reticulata* (Orbigny) and *Cymbulia peroni* De Blainville is given following the nomenclature of Levan *et al.* (1964) after chromosome measurements.

The chromosomal features among Thecosomata could be assigned the following trends: (i) from low to high diploid number ($2n=20$ to $2n=28$ in Euthecosomata, and $2n=24$ to 34 in Pseudotheosomata); (ii) towards an increasing proportion of subtelocentric-telocentric chromosomes (zero to five pairs in Euthecosomata, and one to six Pseudotheosomata).

These features are unusual among Opisthobranchia. First, stable chromosome number within a family, or even an order, has been reported in many Opisthobranchia (Patterson, 1969). However, chromosome number variability of $2n=26$ to $2n=34$ have been observed within the Cephalaspidea order (Curini-Galletti, 1985, 1988), and of one bivalent within the Aplysiidae family (Vitturi *et al.*, 1982b) and

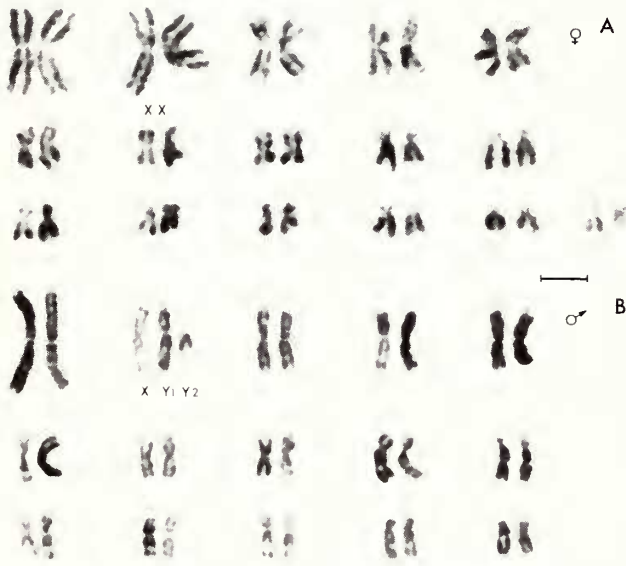


Fig. 6. Karyotypes of *Pterotrachea coronata* (scale bar = 5 μ m), (A), female, (B) male.

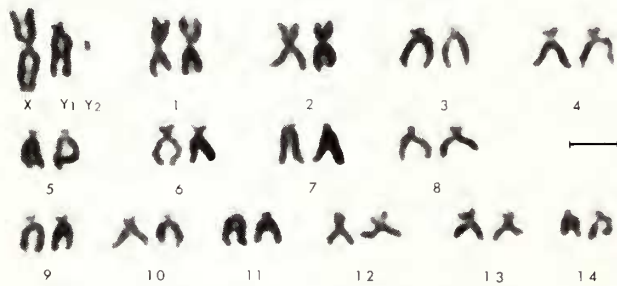


Fig. 7. Male karyotype of *Pterotrachea hippocampus* (scale bar = 5 μ m).

two Soleolifera families (see references in Patterson, 1969). Thus, the chromosome variability occurring among Thecosomata is especially large. Secondly, among the few karyological data recorded among Opisthobranchia, isobrachial chromosomes (i.e. metacentric or submetacentric) of a relatively homogeneous size are dominant in karyotypes (Curini-Galletti, 1988). Hence, Thecosomata differ greatly from the known Opisthobranchia. Moreover, if we assume that isobrachial chromosomes are plesiomorphic as suggested by Curini-Galletti (1988), the karyotypes of *Limacina inflata* showing only metacentric chromosomes should be considered as the most primitive. This point corresponds to the general agreement that the spirally coiled genus *Limacina* is the most primitive of the Euthecosomata (Lalli and Gilmer, 1989).

The evolutionary cytotaxonomy within Thecosomata could be from *Limacina inflata* with low diploid number and metacentric chromosomes to *Cymbulia peroni* with high diploid number and subtelocentric-telocentric chromosomes. The Thecosomata constitutes an isolated order with no karyological relationship with the other opisthobranch orders

and speculation about which ancestral molluscs might have given rise to the Thecosomata cannot be made.

GYMNOSOMATA

The two species studied in this paper share the same diploid number ($2n=32$) as the species previously investigated (Thiriot-Quiévreux, 1988). This supports a chromosome number stability within Gymnosomata. This number is close to the opisthobranch ancestral number of $2n=34$ suggested by Schmeckel (1985). *Pneumodermopsis paucidens* reveals contradictory evolutionary features showing a plesiomorphic character (according to Curini-Galletti, 1988) with its highest proportion of isobrachial chromosomes, but also an apomorphic character (Thiriot-Quiévreux, 1988 according to the hypothesis of Gold and Amemiya, 1986) with the presence of NORs on a metacentric chromosome. *Paraclione longicaudata* differs from the other species by its striking variability in relative length of the different chromosomes and by its high number of subtelocentric chromosomes.

Therefore, karyological features among Gymnosomata are characterized by modal chromosome number of $2n=32$ and a variable proportion between metacentric-submetacentric and subtelocentric-telocentric chromosomes which, at present, preclude evolutionary implications.

In conclusion, karyological analyses of the two orders, Thecosomata and Gymnosomata, lend support for a biphyletical origin, i.e. different opisthobranch ancestors, as generally accepted (see Van der Spoel, 1967, and Lalli and Gilmer, 1989).

HETEROPODA

Since Boveri (1890) reported 32 chromosomes in the females of *Carinaria mediterranea* Blainville and *Pterotrachea mutica* Lesueur (= *P. hippocampus*), nothing has been published on the chromosomes of Heteropoda. The present data confirm a diploid complement of $2n=32$ in the females of *P. coronata* and *Firoloida desmaresti*. But the male diploid complement in the three species of Pterotracheidae here studied consists of $2n=31$ with heteromorphic chromosomes suggesting a XY_1Y_2 sex mechanism. The remaining autosome pairs of the Heteropoda karyotypes show a variable proportion of the different morphological types of chromosomes within the three species studied. The pelagic group of Heteropoda is considered as a superfamily (Boss, 1982), a suborder (Lalli and Gilmer, 1989) or an order (Van der Spoel, 1976) of the Prosobranchia Mesogastropoda. Chromosome number of $2n=14$ to $2n=34$ has been reported within Mesogastropoda, excluding polyploid species of Cerithiacea (see Patterson, 1969). In recent studies on Mesogastropoda, a chromosome number of $2n=32$ in Rissoidae (Thiriot-Quiévreux and Ayraud, 1982), $2n=34$ in Littorinidae (Janson, 1983; Vitturi et al., 1986, 1988) and in Naticidae (Vitturi et al., 1982a; Komatsu, 1985) has been found. In the hydrobioid *Tricula aperta* (Temcharoen), diploid chromosome number showed variations from $2n=32$ to 34 in females and $2n=31$ to 33 in males (Kitikoon, 1982). Thus, the diploid complement observed in Heteropoda ranges close to these numbers.

Table 4. List of known karyological data for pelagic gastropods.

	Diploid no.	sex chromo.	Morphological types (no. of chromosome pairs of autosomes)					
			m	sm	sm-st st-sm	st	t	
Opisthobranchia								
Thecosomata, Euthecosomata								
<i>Limacina inflata</i>	20		10					
<i>Creseis acicula</i> (Rang)	20		5	5				
<i>Creseis virgula</i> (Rang)	20		7	1	2			
<i>Clio pyramidata</i> Linné	22		5	1	2	2	1	
<i>Cavolinia inflexa</i> (Lesueur)	24		6	1	2	3		
<i>Hyalocylis striata</i>	28		7	2	1	4		
Thecosomata, Pseudothecosomata								
<i>Peraclis reticulata</i>	24		6	4	1	1		
<i>Cymbulia peroni</i>	34		5	2	4	5	1	
Gymnosomata								
<i>Pneumodermopsis canephora</i>	32		2	3	5	5	1	
<i>Pneumodermopsis paucidens</i>	32		7	6	1	1	1	
<i>Pneumoderma atlanticum</i>	32		3	5	4	4		
<i>Paraclione longicaudata</i>	32		4	2	1	7	2	
Prosobranchia								
Heteropoda								
<i>Pterotrachea coronata</i>	female	32	XX	7	5	1	2	
	male	31	XY ₁ Y ₂	7	4	1	2	
<i>Pterotrachea hippocampus</i>	male	31	XY ₁ Y ₂	2	2	1	6	3
<i>Firoloida desmaresti</i>	female	32	XX	4	4		5	2
	male	31	XY ₁ Y ₂	4	4		4	2

m : metacentric; sm : submetacentric; sm-st and st-sm : 23.06 < Ci > 26.74; st : subtelocentric; t : telocentric.

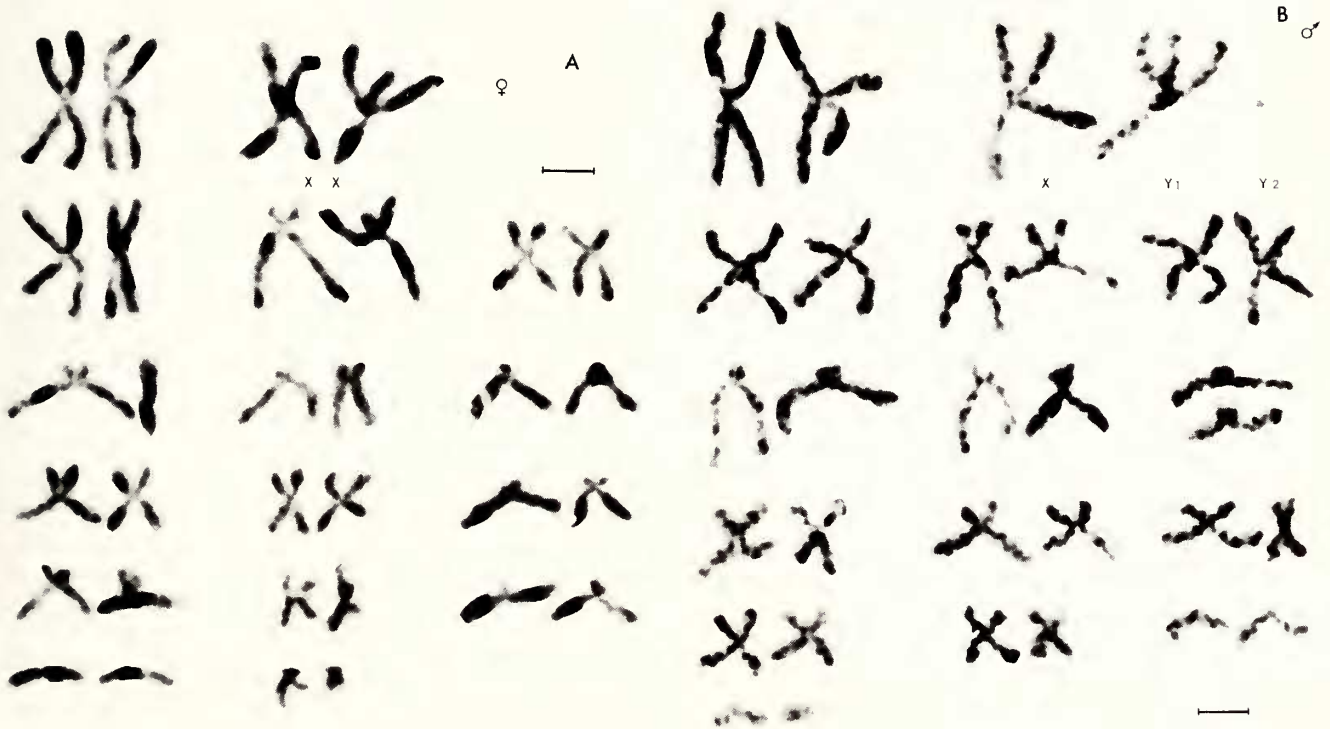


Fig. 8. Karyotypes of *Firoloida desmaresti* (scale bar = 5 μm), (A) female, (B) male.

Sex chromosomes have been observed in several species of Mesogastropoda with difference in male sex-determining mechanisms (YX vs. XO) (see references in Patterson, 1969; and Stern, 1975; Thiriou-Quévieux, 1982; Kitikoon, 1982; Vitturi *et al.*, 1988). Therefore, the XY₁Y₂ male sex mechanism here suggested in Pterotracheidae is very unusual among molluscs and probably involves translocations between sex chromosomes and autosomes (White, 1973). This sex mechanism, even if Y₂ is a microchromosome as in *Firoloida desmaresti* suggests a multiple sex-chromosome mechanism as reported by White (1973) in different taxons of the animal kingdom. However, further investigations on males and females of Heteropoda need to be carried out to confirm and elucidate this sex mechanism.

ACKNOWLEDGMENTS

Thanks are due to R. Voelksen for providing live animals from the plankton, to G. Quéart for her excellent technical assistance and to P. Chang for English corrections.

LITERATURE CITED

- Boss, R. T. 1982. Mollusca. In: *Synopsis and classification of living organisms*, S. Q. Parker, ed. pp. 1092-1096. McGraw-Hill, New York.
- Boveri, T. 1890. Zellen Studien. Über das Verhalten der chromatischen Kernsubstanz bei der Bildung der Richtungkörper und bei der Befruchtung. *Jenaische Zeitschrift für Naturwissenschaft* 24:314-401.
- Curini-Galletti, M. C. 1985. Chromosome morphology of *Philinoglossa praelongata* (Gastropoda Cephalaspidea). *Journal of Molluscan Studies* 51:220-222.
- Curini-Galletti, M. C. 1988. Analyse du caryotype de *Runcina coronata* (Gastropoda Cephalaspidea). *Cahiers de Biologie Marine* 29:313-318.
- Gold, J. R. and C. T. Amemiya. 1986. Cytogenetic studies in North American minnows (Cyprinidae). XII. Pattern of chromosomal nucleolus organizer region variation among 14 species. *Canadian Journal of Zoology* 64:1869-1877.
- Howell, W. H. and D. A. Black. 1980. Controlled silver-staining of nucleolus organizer regions with a protective colloidal developer: a 1-step method. *Experientia* 36:1014-1015.
- Janson, K. 1983. Chromosome number in two phenotypically distinct populations of *Littorina saxatilis* Olivi and in specimens of the *Littorina obtusata* (L.) species complex. *Journal of Molluscan Studies* 49:224-227.
- Kitikoon, V. 1982. Studies on *Tricola aperta* and related taxa, the snail intermediate hosts of *Schistosoma mekongi*. IV. Chromosome studies. *Malacological Review* 15:21-42.
- Komatsu, S. 1985. Karyotypes of two species in two families of Prosobranchia. *Venus (Japanese Journal of Malacology)* 44:49-54.
- Lalli, C. M. and R. W. Gilmer. 1989. *Pelagic snails*. Stanford University Press, Stanford, California. 259 pp.
- Levan, A., K. Fredga and A. A. Sanders. 1964. Nomenclature for centromeric position on chromosomes. *Hereditas* 52:201-220.
- Nakamura, H. K. 1985. A review of molluscan cytogenetic information based on the CISMOCH-computerized system for molluscan chromosomes. Bivalvia, Polyplacophora and Cephalopoda. *Venus (Japanese Journal of Malacology)* 44:193-225.
- Nakamura, H. K. 1986. Chromosomes of Archaeogastropoda (Mollusca Prosobranchia) with some remarks on their cytotaxonomy and phylogeny. *Publications of the Seto Marine Biological Laboratory* 31:191-267.
- Nakrassov, A. 1904. Untersuchung über die Reifung und Befruchtung des Eies von *Cymbulia peroni*. *Anatomisches Anzeiger* 24:119-127.
- Patterson, C. M. 1969. Chromosomes of molluscs. In: *Proceedings of 2nd symposium on Mollusca*. Ernakulam, Cochin, India. Vol. 2. pp. 635-689. Marine Biological Association of India, Ramanathapuram District, Madras State.
- Schmeckel, L. 1985. Aspects of evolution within the opisthobranchs. In: *The Mollusca*. E. R. Trueman and M. R. Clarke, eds. pp. 221-267. Academic Press, New York.
- Stern, E. M. 1975. The chromosomes of *Viviparus viviparus* (Say) (Streptoneura: Viviparidae). *Malacological Review* 8:107-108.
- Thiriou-Quévieux, C. 1984. Chromosome analysis of *Mytilus* (Bivalvia Mytilidae). *Marine Biology Letters* 5:265-273.
- Thiriou-Quévieux, C. 1988. Chromosome studies in pelagic opisthobranch molluscs. *Canadian Journal of Zoology* 66:1460-1473.
- Thiriou-Quévieux, C. and N. Ayraud. 1982. Les caryotypes de quelques espèces de Bivalves et de Gastéropodes marins. *Marine Biology* 70:165-172.
- Thiriou-Quévieux, C., J. Soyer, F. de Bovée, and P. Albert. 1988. Unusual chromosome complement in the brooding bivalve *Lasaea consanguinea*. *Genetica* 76:143-151.
- Van der Spoel, S. 1967. *Euthecosomata*, a group with remarkable developmental stages (Gastropoda Pteropoda). J. Norduun en Zoon, N. V., Gorinchem, Netherlands. 375 pp.
- Van der Spoel, S. 1976. *Pseudothecosomata*, *Gymnosomata* and *Heteropoda* (Gastropoda). Bohn, Scheltema & Holkema, Evren, B. V., Utrecht. 484 pp.
- Vitturi, R., M. B. Rasotto, and N. Farinella-Ferruzza. 1982a. The chromosomes of 16 molluscan species. *Bollettino di Zoologia* 49:61-71.
- Vitturi, R., M. Rasotto, N. Parrinello, and E. Catalano. 1982b. Spermatocyte chromosomes in some species of the family Aplysiidae (Gastropoda, Opisthobranchia). *Caryologia* 35:327-333.
- Vitturi, R., E. Catalano, and M. Macaluso. 1986. Chromosome studies in three species of the gastropod family Littorinidae. *Malacological Review* 19:53-60.
- Vitturi, R., E. Catalano, M. Macaluso, and B. Zava. 1988. The karyology of *Littorina neritoides* Linnaeus, 1758) (Mollusca, Prosobranchia). *Malacologia* 29(2):319-324.
- White, H. S. D. 1973. *Animal cytology and evolution*. 3rd ed. Cambridge University Press. 961 pp.
- Zarnick, B. 1911. Über den Chromosomenzyklus bei Pteropoden. *Verhandlungen der Deutschen Zoologischen Gesellschaft* 20:205-215.

Date of manuscript acceptance: 2 November 1989